Simazine
Analysis of Risks
to
Endangered and Threatened Salmon and Steelhead

July 25, 2003

Larry Turner, Ph.D.
Environmental Field Branch
Office of Pesticide Programs

Summary

Simazine is a selective herbicide used on various crops and non-crop sites. It is slightly to moderately toxic to fish and aquatic invertebrates, and it is highly toxic to aquatic vascular plants. Modeling of potential simazine estimated environmental concentrations (EECs) indicates that with the highest application rates, the most vulnerable soils, and a very high runoff potential, the upper tenth percentile EECs do not exceed any levels of concern. Federally listed Pacific salmon and steelhead occur in areas with lower application rates, typically less vulnerable soils, and less runoff potential, thus providing even a greater margin of safety than would result from the modeled EECs. I conclude that there will be no effects on any ESU of listed Pacific salmon or steelhead.

Introduction

This analysis was prepared by the U.S. Environmental Protection Agency (EPA) Office of Pesticides (OPP) to evaluate the risks of simazine to threatened and endangered Pacific salmon and steelhead. The format of this analysis is the same as for previous analyses. The background section explaining the risk assessment process is the same as was presented in a previous assessment for diazinon, except that we have modified our criterion of concern for indirect effects on aquatic vegetation as cover for listed fish. Previously, our Level of Concern ((LOC) was 0.5 times the aquatic plant (preferably vascular plant) EC50. The intent for indirect effects is to provide protection for populations of species that serve as food or cover for listed species. We have attempted to maintain consistency with the standard risk assessment procedures used by the Environmental Fate and Effects Division (EFED), but there were changes in the criteria for plants that have not been previously captured. The acute risk concern for aquatic plant populations in EFED is now the EC50, without any additional factor. This is now reflected in the criteria in the background section.

Unlike most other pesticides being evaluated for effects on salmon and steelhead, a Reregistration Eligibility Decision (RED) document has not yet been prepared, and it is not scheduled for development until after legal deadlines for our simazine analysis. Certain components have been developed, but these have not been made available for review and are considered draft documents. Therefore, we have extracted information from a variety of individual pieces in EFED files. As usual, we have also extracted toxicity data from EFED's one-liner data base. In addition, we have looked at relevant literature.

Problem Formulation - The purpose of this analysis is to determine whether the registration of simazine as an herbicide for use on various crops and nonagricultural sites may affect threatened and endangered (T&E or listed) Pacific anadromous salmon and steelhead and their designated critical habitat.

Scope - This analysis is specific to listed western salmon and steelhead and the watersheds in which they occur. It is acknowledged that the use of simazine occurs outside this geographic scope and that additional analyses may be required to address other T&E species in the Pacific states as well as across the United States.

Contents

1. Background

Under section 7 of the Endangered Species Act, the Office of Pesticide Programs (OPP) of the U. S. Environmental Protection Agency (EPA) is required to consult on actions that 'may affect' Federally listed endangered or threatened species or that may adversely modify designated critical habitat. Situations where a pesticide may affect a fish, such as any of the salmonid species listed by the National Marine Fisheries Service (NMFS), include either direct or indirect effects on the fish. Direct effects result from exposure to a pesticide at levels that may cause harm.

Acute Toxicity - Relevant acute data are derived from standardized toxicity tests with lethality as the primary endpoint. These tests are conducted with what is generally accepted as the most sensitive life stage of fish, i.e., very young fish from 0.5-5 grams in weight, and with species that are usually among the most sensitive. These tests for pesticide registration include analysis of observable sublethal effects as well. The intent of acute tests is to statistically derive a median effect level; typically the effect is lethality in fish (LC50) or immobility in aquatic invertebrates (EC50). Typically, a standard fish acute test will include concentrations that cause no mortality, and often no observable sublethal effects, as well as concentrations that would cause 100% mortality. By looking at the effects at various test concentrations, a dose-response curve can be derived, and one can statistically predict the effects likely to occur at various pesticide concentrations; a well done test can even be extrapolated, with caution, to concentrations below those tested (or above the test concentrations if the highest concentration did not produce 100% mortality).

OPP typically uses qualitative descriptors to describe different levels of acute toxicity, the most likely kind of effect of modern pesticides (Table 1). These are widely used for comparative purposes, but must be associated with exposure before any conclusions can be drawn with respect to risk. Pesticides that are considered highly toxic or very highly toxic are required to have a label statement indicating that level of toxicity. The FIFRA regulations [40CFR158.490(a)] do not require calculating a specific LC50 or EC50 for pesticides that are practically non-toxic; the LC50 or EC50 would simply be expressed as >100 ppm. When no lethal or sublethal effects are observed at 100 ppm, OPP considers the pesticide will have "no effect" on the species.

Table 1. Qualitative descriptors for categories of fish and aquatic invertebrate toxicity (from Zucker, 1985)

LC50 or EC50	Category description
< 0.1 ppm	Very highly toxic
0.1- 1 ppm	Highly toxic
>1 < 10 ppm	Moderately toxic
> 10 < 100 ppm	Slightly toxic
> 100 ppm	Practically non-toxic

Comparative toxicology has demonstrated that various species of scaled fish generally have equivalent sensitivity, within an order of magnitude, to other species of scaled fish tested under the same conditions. Sappington et al. (2001), Beyers et al. (1994) and Dwyer et al. (1999), among others, have shown that endangered and threatened fish tested to date are similarly sensitive, on an acute basis, to a variety of pesticides and other chemicals as their non-endangered counterparts.

Chronic Toxicity - OPP evaluates the potential chronic effects of a pesticide on the basis of several types of tests. These tests are often required for registration, but not always. If a pesticide has essentially no acute toxicity at relevant concentrations, or if it degrades very rapidly in water, or if the nature of the use is such that the pesticide will not reach water, then chronic fish tests may not be required [40CFR158.490]. Chronic fish tests primarily evaluate the potential for reproductive effects and effects on the offspring. Other observed sublethal effects are also required to be reported. An abbreviated chronic test, the fish early-life stage test, is usually the first chronic test conducted and will indicate the likelihood of reproductive or chronic effects at relevant concentrations. If such effects are found, then a full fish life-cycle test will be conducted. If the nature of the chemical is such that reproductive effects are expected, the abbreviated test may be skipped in favor of the full life-cycle test. These chronic tests are designed to determine a “no observable effect level” (NOEL) and a “lowest observable effect level” (LOEL). A chronic risk requires not only chronic toxicity, but also chronic exposure, which can result from a chemical being persistent and resident in an environment (e.g., a pond) for a chronic period of time or from repeated applications that transport into any environment such that exposure would be considered “chronic”.

As with comparative toxicology efforts relative to sensitivity for acute effects, EPA, in conjunction with the U. S. Geological Survey, has a current effort to assess the comparative toxicology for chronic effects also. Preliminary information indicates, as with the acute data, that endangered and threatened fish are again of similar sensitivity to similar non-endangered species.

Metabolites and Degradates - Information must be reported to OPP regarding any pesticide metabolites or degradates that may pose a toxicological risk or that may persist in the environment [40CFR159.179]. Toxicity and/or persistence test data on such compounds may be required if, during the risk assessment, the nature of the metabolite or degradate and the amount that may occur in the environment raises a concern. If actual data or structure-activity analyses are not available, the requirement for testing is based upon best professional judgement.

Inert Ingredients - OPP does take into account the potential effects of what used to be termed “inert” ingredients, but which are beginning to be referred to as “other ingredients”. OPP has classified these ingredients into several categories. A few of these, such as nonylphenol, can no longer be used without including them on the label with a specific statement indicating the potential toxicity. Based upon our internal databases, I can find no product in which nonylphenol is now an ingredient. Many others, including such ingredients as clay, soybean oil, many polymers, and chlorophyll, have been evaluated through structure-activity analysis or data and determined to be of minimal or no toxicity. There exist also two additional lists, one for inerts with potential toxicity which are considered a testing priority, and one for inerts unlikely to be toxic, but which cannot yet be said to have negligible toxicity. Any new inert ingredients are required to undergo testing unless it can be demonstrated that testing is unnecessary.

The inerts efforts in OPP are oriented only towards toxicity at the present time, rather than risk. It should be noted, however, that very many of the inerts are in exceedingly small amounts in pesticide products. While some surfactants, solvents, and other ingredients may be present in fairly large amounts in various products, many are present only to a minor extent. These include such things as coloring agents, fragrances, and even the printers ink on water soluble bags of pesticides. Some of these could have moderate toxicity, yet still be of no consequence because of the negligible amounts present in a product. If a product contains inert ingredients in sufficient quantity to be of concern, relative to the toxicity of the active ingredient, OPP attempts to evaluate the potential effects of these inerts through data or structure-activity analysis, where necessary.

For a number of major pesticide products, testing has been conducted on the formulated end-use products that are used by the applicator. The results of fish toxicity tests with formulated products can be compared with the results of tests on the same species with the active ingredient only. A comparison of the results should indicate comparable sensitivity, relative to the percentage of active ingredient in the technical versus formulated product, if there is no extra activity due to the combination of inert ingredients. I note that the "comparable" sensitivity must take into account the natural variation in toxicity tests, which is up to 2-fold for the same species in the same laboratory under the same conditions, and which can be somewhat higher between different laboratories, especially when different stocks of test fish are used.

The comparison of formulated product and technical ingredient test results may not provide specific information on the individual inert ingredients, but rather is like a "black box" which sums up the effects of all ingredients. I consider this approach to be more appropriate than testing each individual inert and active ingredient because it incorporates any additivity, antagonism, and synergism effects that may occur and which might not be correctly evaluated from tests on the individual ingredients. I do note, however, that we do not have aquatic data on most formulated products, although we often have testing on one or perhaps two formulations of an active ingredient.

Risk - An analysis of toxicity, whether acute or chronic, lethal or sublethal, must be combined with an analysis of how much will be in the water, to determine risks to fish. Risk is a combination of exposure and toxicity. Even a very highly toxic chemical will not pose a risk if there is no exposure, or very minimal exposure relative to the toxicity. OPP uses a variety of chemical fate and transport data to develop "estimated environmental concentrations" (EECs) from a suite of established models. The development of aquatic EECs is a tiered process.

The first tier screening model for EECs is with the GENEEC program, developed within OPP, which uses a generic site (in Yazoo, MS) to stand for any site in the U. S. The site choice was intended to yield a maximum exposure, or "worst-case," scenario applicable nationwide, particularly with respect to runoff. The model is based on a 10 hectare watershed that surrounds a one hectare pond, two meters deep. It is assumed that all of the 10 hectare area is treated with the pesticide and that any runoff would drain into the pond. The model also incorporates spray drift, the amount of which is dependent primarily upon the droplet size of the spray. OPP assumes that if this model indicates no concerns when compared with the appropriate toxicity data, then further analysis is not necessary as there would be no effect on the species.

It should be noted that prior to the development of the GENEEC model in 1995, a much more crude approach was used to determining EECs. Older reviews and Reregistration Eligibility Decisions (REDs) may use this approach, but it was excessively conservative and does not provide a sound basis for modern risk assessments. For the purposes of endangered species consultations, we will attempt to revise this old approach with the GENEEC model, where the old screening level raised risk concerns.

When there is a concern with the comparison of toxicity with the EECs identified in GENEEC model, a more sophisticated PRZM-EXAMS model is run to refine the EECs if a suitable scenario has been developed and validated. The PRZM-EXAMS model was developed with widespread collaboration and review by chemical fate and transport experts, soil scientists, and agronomists throughout academia, government, and industry, where it is in common use. As with the GENEEC model, the basic model remains as a 10 hectare field surrounding and draining into a 1 hectare pond. Crop scenarios have been developed by OPP for specific sites, and the model uses site-specific data on soils, climate (especially precipitation), and the crop or site. Typically, site-scenarios are developed to provide for a worst-case analysis for a particular crop in a particular geographic region. The development of site scenarios is very time consuming; scenarios have not yet been developed for a number of crops and locations. OPP attempts to match the crop(s) under consideration with the most appropriate scenario. For some of the older OPP analyses, a very limited number of scenarios were available.

One area of significant weakness in modeling EECs relates to residential uses, especially by homeowners, but also to an extent by commercial applicators. There are no usage data in OPP that relate to pesticide use by homeowners on a geographic scale that would be appropriate for an assessment of risks to listed species. For example, we may know the maximum application rate for a lawn pesticide, but we do not know the size of the lawns, the proportion of the area in lawns, or the percentage of lawns that may be treated in a given geographic area. There is limited information on soil types, slopes, watering practices, and other aspects that relate to transport and fate of pesticides. We do know that some homeowners will attempt to control pests with chemicals and that others will not control pests at all or will use non-chemical methods. We would expect that in some areas, few homeowners will use pesticides, but in other areas, a high percentage could. As a result, OPP has insufficient information to develop a scenario or address the extent of pesticide use in a residential area.

It is, however, quite necessary to address the potential that home and garden pesticides may have to affect T&E species, even in the absence of reliable data. Therefore, I have developed a hypothetical scenario, by adapting an existing scenario, to address pesticide use on home lawns where it is most likely that residential pesticides will be used outdoors. It is exceedingly important to note that there is no quantitative, scientifically valid support for this modified scenario; rather it is based on my best professional judgement. I do note that the original scenario, based on golf course use, does have a sound technical basis, and the home lawn scenario is effectively the same as the golf course scenario. Three approaches will be used. First, the treatment of fairways, greens, and tees will represent situations where a high proportion of homeowners may use a pesticide. Second, I will use a 10% treatment to represent situations where only some homeowners may use a pesticide. Even if OPP cannot reliably determine the percentage of homeowners using a pesticide in a given area, this will provide two estimates. Third, where the risks from lawn use could exceed our criteria by only a modest amount, I can back-calculate the percentage of land that would need to be treated to exceed our criteria. If a smaller percentage is treated, this would then be below our criteria of concern. The percentage here would be not just of lawns, but of all of the treatable area under consideration; but in urban and highly populated suburban areas, it would be similar to a percentage of lawns. Should reliable data or other information become available, the approach will be altered appropriately.

It is also important to note that pesticides used in urban areas can be expected to transport considerable distances if they should run off on to concrete or asphalt, such as with streets (e.g., TDK Environmental, 1991). This makes any quantitative analysis very difficult to address aquatic exposure from home use. It also indicates that a no-use or no-spray buffer approach for protection, which we consider quite viable for agricultural areas, may not be particularly useful for urban areas.

Finally, the applicability of the overall EEC scenario, i.e., the 10 hectare watershed draining into a one hectare farm pond, may not be appropriate for a number of T&E species living in rivers or lakes. This scenario is intended to provide a "worst-case" assessment of EECs, but very many T&E fish do not live in ponds, and very many T&E fish do not have all of the habitat surrounding their environment treated with a pesticide. OPP does believe that the EECs from the farm pond model do represent first order streams, such as those in headwaters areas (Effland, et al. 1999). In many agricultural areas, those first order streams may be upstream from pesticide use, but in other areas, or for some non-agricultural uses such as forestry, the first order streams may receive pesticide runoff and drift. However, larger streams and lakes will very likely have lower, often considerably lower, concentrations of pesticides due to more dilution by the receiving waters. In addition, where persistence is a factor, streams will tend to carry pesticides away from where they enter into the streams, and the models do not allow for this. The variables in size of streams, rivers, and lakes, along with flow rates in the lotic waters and seasonal variation, are large enough to preclude the development of applicable models to represent the diversity of T&E species' habitats. We can simply qualitatively note that the farm pond model is expected to overestimate EECs in larger bodies of water.

Indirect Effects - We also attempt to protect listed species from indirect effects of pesticides. We note that there is often not a clear distinction between indirect effects on a listed species and adverse modification of critical habitat (discussed below). By considering indirect effects first, we can provide appropriate protection to listed species even where critical habitat has not been designated. In the case of fish, the indirect concerns are routinely assessed for food and cover.

The primary indirect effect of concern would be for the food source for listed fish. These are best represented by potential effects on aquatic invertebrates, although aquatic plants or plankton may be relevant food sources for some fish species. However, it is not necessary to protect individual organisms that serve as food for listed fish. Thus, our goal is to ensure that pesticides will not impair populations of these aquatic arthropods. In some cases, listed fish may feed on other fish. Because our criteria for protecting the listed fish species is based upon the most sensitive species of fish tested, then by protecting the listed fish species, we are also protecting the species used as prey.

In general, but with some exceptions, pesticides applied in terrestrial environments will not affect the plant material in the water that provides aquatic cover for listed fish. Application rates for herbicides are intended to be efficacious, but are not intended to be excessive. Because only a portion of the effective application rate of an herbicide applied to land will reach water through runoff or drift, the amount is very likely to be below effect levels for aquatic plants. Some of the applied herbicides will degrade through photolysis, hydrolysis, or other processes. In addition, terrestrial herbicide applications are efficacious in part, due to the fact that the product will tend to stay in contact with the foliage or the roots and/or germinating plant parts, when soil applied. With aquatic exposures resulting from terrestrial applications, the pesticide is not placed in immediate contact with the aquatic plant, but rather reaches the plant indirectly after entering the water and being diluted. Aquatic exposure is likely to be transient in flowing waters. However, because of the exceptions where terrestrially applied herbicides could have effects on aquatic plants, OPP does evaluate the sensitivity of aquatic macrophytes to these herbicides to determine if populations of aquatic macrophytes that would serve as cover for T&E fish would be affected.

For most pesticides applied to terrestrial environment, the effects in water, even lentic water, will be relatively transient. Therefore, it is only with very persistent pesticides that any effects would be expected to last into the year following their application. As a result, and excepting those very persistent pesticides, we would not expect that pesticidal modification of the food and cover aspects of critical habitat would be adverse beyond the year of application. Therefore, if a listed salmon or steelhead is not present during the year of application, there would be no concern. If the listed fish is present during the year of application, the effects on food and cover are considered as indirect effects on the fish, rather than as adverse modification of critical habitat.

Designated Critical Habitat - OPP is also required to consult if a pesticide may adversely modify designated critical habitat. In addition to the indirect effects on the fish, we consider that the use of pesticides on land could have such an effect on the critical habitat of aquatic species in a few circumstances. For example, use of herbicides in riparian areas could affect riparian vegetation, especially woody riparian vegetation, which possibly could be an indirect effect on a listed fish. However, there are very few pesticides that are registered for use on riparian vegetation, and the specific uses that may be of concern have to be analyzed on a pesticide by pesticide basis. In considering the general effects that could occur and that could be a problem for listed salmonids, the primary concern would be for the destruction of vegetation near the stream, particularly vegetation that provides cover or temperature control, or that contributes woody debris to the aquatic environment. Destruction of low growing herbaceous material would be a concern if that destruction resulted in excessive sediment loads getting into the stream, but such increased sediment loads are insignificant from cultivated fields relative to those resulting from the initial cultivation itself. Increased sediment loads from destruction of vegetation could be a concern in uncultivated areas. Any increased pesticide load as a result of destruction of terrestrial herbaceous vegetation would be considered a direct effect and would be addressed through the modeling of estimated environmental concentrations. Such modeling can and does take into account the presence and nature of riparian vegetation on pesticide transport to a body of water.

Risk Assessment Processes - All of our risk assessment procedures, toxicity test methods, and EEC models have been peer-reviewed by OPP's Science Advisory Panel. The data from toxicity tests and environmental fate and transport studies undergo a stringent review and validation process in accordance with "Standard Evaluation Procedures" published for each type of test. In addition, all test data on toxicity or environmental fate and transport are conducted in accordance with Good Laboratory Practice (GLP) regulations (40 CFR Part 160) at least since the GLPs were promulgated in 1989.

The risk assessment process is described in "Hazard Evaluation Division - Standard Evaluation Procedure - Ecological Risk Assessment" by Urban and Cook (1986) (termed

Ecological Risk Assessment SEP below), which has been separately provided to National Marine Fisheries Service staff. Although certain aspects and procedures have been updated throughout the years, the basic process and criteria still apply. In a very brief summary: the toxicity information for various taxonomic groups of species is quantitatively compared with the potential exposure information from the different uses and application rates and methods. A risk quotient of toxicity divided by exposure is developed and compared with criteria of concern. The criteria of concern presented by Urban and Cook (1986) are presented in Table 2.

Table 2. Risk quotient criteria for fish and for direct and indirect effects on T&E fish

Test data	Risk quotient	Presumption
Acute LC50	>0.5	Potentially high acute risk
Acute LC50	>0.1	Risk that may be mitigated through restricted use classification
Acute LC50	>0.05	Endangered species may be affected acutely, including sublethal effects
Chronic NOEC	>1	Chronic risk; endangered species may be affected chronically, including reproduction and effects on progeny
Acute invertebrate LC50[a]	>0.5	May be indirect effects on T&E fish through food supply reduction
Aquatic plant acute EC50[a]	>1[b]	May be indirect effects on aquatic vegetative cover for T&E fish

a. Indirect effects criteria for T&E species are not in Urban and Cook (1986); they were developed subsequently.
b. This criterion has been changed from previous requests. The basis is to bring the endangered species criterion for indirect effects on aquatic plant populations in line with EFED's concern levels for these populations..

The Ecological Risk Assessment SEP (pages 2-6) discusses the quantitative estimates of how the acute toxicity data, in combination with the slope of the dose-response curve, can be used to predict the percentage mortality that would occur at the various risk quotients. The discussion indicates that using a "safety factor" of 10, as applies for restricted use classification, one individual in 30,000,000 exposed to the concentration would be likely to die. Using a "safety factor" of 20, as applies to aquatic T&E species, would exponentially increase the margin of safety. It has been calculated by one pesticide registrant (without sufficient information for OPP to validate that number), that the probability of mortality occurring when the LC50 is 1/20th of the EEC is 2.39×10^{-9}, or less than one individual in ten billion. It should be noted that the discussion (originally part of the 1975 regulations for FIFRA) is based upon slopes of primarily organochlorine pesticides, stated to be 4.5 probits per log cycle at that time. As organochlorine pesticides were phased out, OPP undertook an analysis of more current pesticides based on data reported by Johnson and Finley (1980), and determined that the "typical" slope for aquatic toxicity tests for the "more current" pesticides was 9.95. Because the slopes are based upon logarithmically transformed data, the probability of mortality for a pesticide with a 9.95 slope is again exponentially less than for the originally analyzed slope of 4.5.

The above discussion focuses on mortality from acute toxicity. OPP is concerned about other direct effects as well. For chronic and reproductive effects, our criteria ensures that the EEC is below the no-observed-effect-level, where the "effects" include any observable sublethal effects. Because our EEC values are based upon "worst-case" chemical fate and transport data and a small farm pond scenario, it is rare that a non-target organism would be exposed to such concentrations over a period of time, especially for fish that live in lakes or in streams (best professional judgement). Thus, there is no additional safety factor used for the no-observed-effect-concentration, in contrast to the acute data where a safety factor is warranted because the endpoints are a median probability rather than no effect.

Sublethal Effects - With respect to sublethal effects, Tucker and Leitzke (1979) did an extensive review of existing ecotoxicological data on pesticides. Among their findings was that sublethal effects as reported in the literature did not occur at concentrations below one-fourth to one-sixth of the lethal concentrations, when taking into account the same percentages or numbers affected, test system, duration, species, and other factors. This was termed the "6x hypothesis". Their review included cholinesterase inhibition, but was largely oriented towards externally observable parameters such as growth, food consumption, behavioral signs of intoxication, avoidance and repellency, and similar parameters. Even reproductive parameters fit into the hypothesis when the duration of the test was considered. This hypothesis supported the use of lethality tests for use in assessing ecotoxicological risk, and the lethality tests are well enough established and understood to provide strong statistical confidence, which can not always be achieved with sublethal effects. By providing an appropriate safety factor, the concentrations found in lethality tests can therefore generally be used to protect from sublethal effects.

In recent years, Moore and Waring (1996) challenged Atlantic salmon with diazinon and observed effects on olfaction as relates to reproductive physiology and behavior. Their work indicated that diazinon could have sublethal effects of concern for salmon reproduction. However, the nature of their test system, direct exposure of olfactory rosettes, could not be quantitatively related to exposures in the natural environment. Subsequently, Scholz et al. (2000) conducted a non-reproductive behavioral study using whole Chinook salmon in a model stream system that mimicked a natural exposure that is far more relevant to ecological risk assessment than the system used by Moore and Waring (1996). The Scholz et al. (2000) data indicate potential effects of diazinon on Chinook salmon behavior at very low levels, with statistically significant effects at nominal diazinon exposures of 1 ppb, with apparent, but non-significant effects at 0.1 ppb.

It would appear that the Scholz et al (2000) work contradicts the 6x hypothesis. The research design, especially the nature and duration of exposure, of the test system used by Scholz et al (2000), along with a lack of dose-response, precludes comparisons with lethal levels in accordance with 6x hypothesis as used by Tucker and Leitzke (1979). Nevertheless, it is known that olfaction is an exquisitely sensitive sense. And this sense may be particularly well developed in salmon, as would be consistent with its use by salmon in homing (Hasler and Scholz, 1983). So the contradiction of the 6x hypothesis is not surprising. As a result of these findings, the 6x hypothesis needs to be re-evaluated with respect to olfaction. At the same time, because of the sensitivity of olfaction and because the 6x hypothesis has generally stood the test of time otherwise, it would be premature to abandon the hypothesis for other sublethal effects until there are additional data.

2. Description of simazine

Simazine is an herbicide most frequently used in crops as a pre-emergent or early post-emergent treatment to soil or early vegetation. At typical rates, it is a selective herbicide controlling broad-leaved plants and annual grasses. At higher rates, it can provide non-selective control in such non-crop sites as industrial areas and rights-of-way. Simazine enters target plants primarily through the root system and operates by inhibiting photosynthesis, but apparently causes plant death due to a buildup of reactive chemicals that disrupt cell membranes (Kansas State University, 1990). Non-susceptible plants, such as corn and sorghum, have the ability to metabolize the simazine into substances with no herbicidal activity.

a. Registered uses

Simazine is federally registered as a selective herbicide for use on corn, a variety of fruit and nut crops, artichokes, asparagus, sugarcane, turf grasses including golf courses and lawns, Christmas trees, and ornamentals in nurseries. Not all of these are permitted for use in states with western salmon and steelhead. Special Local Needs (i.e., state) registrations include alfalfa and cabbage in Washington, and broccoli, Brussels sprouts, cabbage, kale, and kohlrabi in Oregon. There are no special local needs registrations in California or Idaho. At higher rates, simazine is registered for non-selective use in industrial sites and rights-of-way, but the very high rates that used to be specified for these sites are no longer allowed. Simazine is also registered as an aquatic herbicide to control algae in ornamental ponds, aquaria, and commercial fisheries (e.g., catfish farms) water systems. Specific registered crop sites in California, Oregon, Washington, and Idaho are presented in Table 3.

Table 3. Registered crop and noncrop sites for simazine use in western salmon and steelhead states

Crop sites		Non-crop sites
almonds	cranberries	ornamental ponds and aquaria
walnuts	blueberries	nursery ornamentals
filberts	raspberries	Christmas trees
citrus	blackberries	rights-of-way
peaches	loganberries	industrial sites:
nectarines	grapes	fence lines
apples	corn	lumber yards
pears	asparagus	petroleum tank farms
sour cherries	artichokes	equipment and fuel storage areas
avocados	strawberries (OR & WA only)	around buildings
olives	broccoli (OR)	
	Brussel sprouts (OR)	
	kohlrabi (OR)	
	kale (OR)	
	cabbage (OR & WA)	
	alfalfa (WA)	

There are currently 36 federal registrations, along with two Special Local Needs registrations for Oregon and three for Washington. Most products have simazine as the only active ingredient. One product is combined with atrazine for use on corn. This product is considered here. Simazine is a minor component in two other products, with prometon as the primary active ingredient along with sodium metaborate and sodium chlorate. These latter two products will be considered when prometon is evaluated.

b. Simazine usage

All pesticide usage estimates are based largely or wholly on past usage. Future usage of any individual pesticide, such as simazine, may or may not be similar. Changes in future usage, relative to past usage, can result from acreage changes based upon marketing conditions for a particular crop, differing pest pressures, from competition with other pesticides, marketing decisions by pesticide registrants, or federal or state regulatory requirements for a specific pesticide. Within these constraints, we believe that past usage does reflect future usage to a large extent.

According to OPP's preliminary Quantitative Use Assessment (QUA) (attachment #1), based on available pesticide survey usage information for the years of 1987 through 1999, the annual weighted average estimate of simazine's total agricultural usage is approximately 4,034,000 pounds active ingredient (a.i.) for 2,479,000 acres treated; the estimated maximum use would be 5,840,000 pounds ai on 3,393,000 acres. Most of the agricultural acreage is treated with 1.8 pounds a.i./A or less per application, except citrus which may be up to 3 lb ai/A. Repeat applications are allowed for most crops, and the total lb ai/A/yr ranges between 1 and 4.6 pounds a.i. per year. Maximum label rates can be higher, although they are not likely to be used unless there is high pest pressure.

For most of the fruit and nut uses on which simazine is registered, between 20 and 40 percent of the crop is typically treated with simazine, with estimated maximums of 30-70%. With the notable exceptions of artichokes, where 39% are typically treated, asparagus, with 19% typically treated, and corn, with 3% typically treated, 1% or less of registered vegetable crops are typically treated, with estimated maximums up to 6%. Of prominence in salmon and steelhead areas are fruits and nuts, including berries. There appears to be very low use on grains, other than corn, anywhere.

In addition to internal estimates, USDA surveyed usage of simazine on fruit crops (USDA 2002) and on nursery/floriculture crops (USDA 2000) in selected states. Information is available at the state level. California is a "selected state" for many of the surveyed crops, but we believe that the county level pesticide use reporting is more useful than statewide surveys. Therefore, we are presenting information only for Oregon and Washington crops from these two surveys (Tables 4 and 5). California information is below in Tables 6 and 7.

Table 4. Estimated usage of simazine on fruit crops in Oregon and Washington.

Site and state	% area applied	# appl	rate/year	total lb ai applied
apples, OR	18	1	1.06	1,700
apples, WA	9	1	2.28	35,000
blackberries, OR	34	1	1.49	3,100
grapes, WA	18	1	0.49	4,200
pears, OR	18	1.5	1.57	4,900
pears, WA	9	1	1.51	3,300
raspberries, OR	43	1.3	2.14	1,600
raspberries, WA	49	1	0.81	3,700

Table 5. Estimated usage of simazine on nursery and floriculture crops in California and Oregon.

Site	CA %[a]	OR %[a]	rate (lb ai)	total lb ai applied in program states[b]
All nursery & floriculture	1	8	1.67	41,700
All nursery	2	11	1.68	40,000
nursery propagation or lining out stock		6	1.73	1,500
broadleaf evergreens	2	11	1.36	600
coniferous evergreens		6	1.76	7,300
deciduous shade trees	7	6	1.47	1,300
deciduous flowering trees		8	1.7	300
deciduous shrubs and other ornamentals		10	2.66	2,600
fruit and nut plants	5	23	2.02	700
Christmas trees	6	6	1.59	25,600
All floriculture	0	1	1.48	1,700
cut flowers	1			NR
flowering plants		1		NR
herbaceous perennials		4		NR
nonproduction areas			1.25	5,900

[a] Percentage of operations using simazine for each individual category (e.g., Christmas trees). We do not know how well this relates to the percentage of acreage that would be treated, but we suspect the percentages are roughly proportional.
[b] The amount applied is for all program states combined, and is not broken down by individual state. Program states for nursery and floriculture crops are CA, FL, MI, OR, PA, TX..

There has been significant use in lawn care and other non-agricultural sites, based upon the QUA. Turf and lawn use is significant (>800,000 lb ai/yr), but is limited to the southeastern U. S. The other non-agricultural uses apply to Pacific salmon and steelhead areas and include 153,000 pounds (combined) for utility and roadside rights-of-way, and greenhouse-nursery operations.

The latest information for California pesticide use is for the year 2001 [URL: http://www.cdpr.ca.gov/docs/pur/purmain.htm]. The reported information to the County Agricultural Commissioners includes pounds used, acres treated for agricultural and certain other uses, and the specific location treated. The pounds and acres are reported to the state, but the specific location information is retained at the county level and is not readily available. Table 6 presents simazine usage over the past nine years in California. Table 7 presents all of the simazine uses in California for 2001.

Table 6. Reported statewide use of simazine in California, 1993-2001, in pounds of active ingredient

1993	1994	1995	1996	1997	1998	1999	2000	2001
957,812	890,353	837,366	839,209	764,586	794,758	696,574	700,588	586,223

Table 7. Reported use of simazine, by crop or site, for 2001 in California.

crop or site	pounds active ingredient used	acres treated
grapes	224,991	231,361
oranges	180,431	82,722
almonds	51,831	96,529
walnut	33,479	28,157
olives	17,250	9,133
avocado	14,831	12,764
lemon	14,593	6,287
peaches	13,131	19,874
nectarines	9,175	14,526
grapefruit	6,745	3,365
pears	4,423	3,020
rights-of-way	4,383	nr
landscape maintenance	2,894	nr
apples	2,823	3,071
uncultivated agriculture	1,299	327
plums	749	843
tangerine	629	338
lettuce	374	202
citrus	364	141
nursery outdoor container plants	280	211
cherries	225	230
forest, timberland	219	101
nursery-outdoor flowers	169	109
structural pest control	155	nr
uncultivated non-agriculture	144	109

pistachio	101	172
apricots	75	75
nursery greenhouse container plants	56	38
research commodity	52	8
alfalfa	50	120
corn (human consumption)	47	26
prunes	36	52
nursery greenhouse flowers	35	25
blueberries	32	22
pomegranate	28	7
ditch bank	21	24
pecan	20	30
animal premises	16	5
boysenberry	15	12
Christmas trees	13	9
water area	8	10
public health	8	nr
nursery outdoor transplants	6	6
corn (forage-fodder)	4	4
raspberry	4	2
wheat	3	35
tangelo	1	22
spinach	<1	4
bok choy	<1	nr
nursery greenhouse transplants	<1	20
state total	586,223	

3. General aquatic risk assessment data for endangered and threatened salmon and steelhead

a. Aquatic toxicity of simazine

(1) Acute toxicity to freshwater fish

The toxicity tables below include all relevant data in EFED files. However, not all simazine data in the literature are presented. There is a considerable amount of aquatic toxicity data of various types, as might be expected for an herbicide that is intended for use in aquatic ecosystems, as well as on terrestrial sites. We have attempted to capture all data indicating toxicity at low levels, as well as the lowest LC50 data on any salmonid species, even if it would not be considered "low." Numerous tests have been conducted and authors have reported LC50 values higher than the solubility limits of simazine. Solubility appears to be 3.5-5 ppm in water. Perhaps as much as 16 ppm can be solubilized or suspended in fresh water through use of solvents (e.g., acetone) or mechanical means. Tests on technical simazine with reported endpoints above these solubility limits are suspect. Formulations may increase the solubilization of simazine to an unknown degree, and therefore, endpoints somewhat above the 16 ppm solubility may be valid for formulated products.

The lowest standard acute LC50 value for technical simazine is 6.4 ppm for fathead minnows. A lower value of 5 ppm exists for the 4G granular formulation, but liquid formulations show considerably less toxicity, with the exception of two reported by Dad and Tripathi (1980) with Asian species. There are numerous tests on the 80% wettable powder which is registered for use to control algae in aquatic systems, including aquaria and breeding ponds with fish. Acute fish toxicity data are presented in Table 3. Use of the term "AQUIRE" in the reference column means that we were unable to obtain the original paper in a timely fashion.

Table 8. Acute toxicity of simazine to freshwater fish.

Species	Scientific name	% a.i.	96-hour LC50 (ppm)	Toxicity Category	Reference
Technical material					
Rainbow trout	*Oncorhynchus mykiss*	98.1	>100	practically non-toxic	EFED (Johnson & Finley, 1980)
Rainbow trout	*Oncorhynchus mykiss*	tech	70.5	slightly toxic	EFED
Rainbow trout	*Oncorhynchus mykiss*	97.6	>10	≤ slightly toxic	EFED
Rainbow trout	*Oncorhynchus mykiss*	tech	> 2.5 (28-day)	≤ moderately toxic	EFED (Bathe et.al., 1975)
Bluegill sunfish	*Lepomis macrochirus*	99.1	16	slightly toxic	EFED
Fathead minnow	*Pimephales promelas*	tech	6.4	moderately toxic	EFED
Fathead minnow	*Pimephales promelas*	98.1	> 10	≤ slightly toxic	EFED (Mayer & Ellersieck, 1986)
Fathead minnow	*Pimephales promelas*	98.1	>100	practically non-toxic	EFED (Johnson & Finley, 1980)
Goldfish	*Carassius auratus.*	99.1	> 32 ppm	≤ slightly toxic	EFED
Black bullhead	*Ameirus melas*	tech	65	slightly toxic	Bathe et.al., 1975
Crucian carp	*Carrasius carrasius*	tech	>100	practically non-toxic	Bathe et.al., 1975
Rainbow trout	*Oncorhynchus mykiss*	tech	>100	practically non-toxic	Bathe et.al., 1975
Perch	*Perca sp.*	tech	90	slightly toxic	Bathe et.al., 1975
Guppy	*Poecilia reticulata*	tech	49	slightly toxic	Bathe et.al., 1975
Goldfish	*Carassius auratus.*	tech	> 40 (48 hr)	≤ slightly toxic	Nishiuchi & Hashimoto, 1969
Medaka	*Oryzias latipes*	tech	> 40 (48 hr)	≤ slightly toxic	Nishiuchi & Hashimoto, 1969

Species	Scientific name	% a.i.	96-hour LC50 (ppm)	Toxicity Category	Reference
Carp	*Cyprinus carpio*	tech	> 40 (48 hr)	$\leq$ slightly toxic	Nishiuchi & Hashimoto, 1969
Formulated product[b]					
Bluegill sunfish	*Lepomis macrochirus*	80WP	100	practically non-toxic	EFED (Johnson & Finley, 1980)
Bluegill sunfish	*Lepomis macrochirus*	90 WDG	>54.2	slightly toxic	Wall, 2003
Bluegill sunfish	*Lepomis macrochirus*	50 WP	35	slightly toxic	EFED
Pumpkinseed	*Lepomis gibbosus*	50 WP	27	slightly toxic	EFED
Redear sunfish	*Lepomis microlophus*	50 WP	54	slightly toxic	EFED
Largemouth bass	*Lepomis macrochirus*	50 WP	46	slightly toxic	EFED
Rainbow trout	*Oncorhynchus mykiss*	80WP	40.5	slightly toxic	EFED
Rainbow trout	*Oncorhynchus mykiss*	80WP	60	slightly toxic	EFED
Rainbow trout	*Oncorhynchus mykiss*	80WP	44.6	slightly toxic	EFED
Rainbow trout	*Oncorhynchus mykiss*	88.6	> 82	$\leq$ slightly toxic	EFED
Rainbow trout	*Oncorhynchus mykiss*	90 WDG	>19.1	slightly toxic	Wall, 2003
Fathead minnow	*Pimephales promelas*	4G	5	moderately toxic	EFED (Mayer & Ellersieck, 1986)
Fathead minnow	*Pimephales promelas*	80WP	510	practically non-toxic	EFED (Mayer & Ellersieck, 1986)
Striped bass	*Morone saxatilis*	80WP	882	practically non-toxic	Bills et al., 1993
Bluntnose minnow	*Pimephales notatus*	50WP	66	slightly toxic	EFED
Emerald shiner	*Notropis atherinoides*	50	>18	$\leq$ slightly toxic	EFED
Channel catfish	*Ictalurus punctatus*	50WP	85	slightly toxic	EFED
Yellow bullhead	*Ictalurus natalis*	50WP	110	practically non-toxic	EFED
teleost	*Labeo rohita*	50	2.5	moderately toxic	Dad & Tripathi, 1980
Major carp	*Cirrhinus mrigala*	50	2.5	moderately toxic	Dad & Tripathi, 1980
teleost	*Mystus vittatus*	50	28.6	slightly toxic	Dad & Tripathi, 1980
Unidentified or inadequately identified material					
Chinook salmon	*Oncorhynchus tshawytscha*	tech?	6.6 (48 hr)	moderately toxic	Bond et. al., 1960
Guppy	*Poecilia reticulata*	F?	3 ppm (72 hr)	moderately toxic	AQUIRE (Tscheu-Schluter, 1976)
Two-spot barb	*Barbus ticto*	F?	5 (16d)	moderately toxic	AQUIRE (Upadhyaya & Rao, 1980)
Two-spot barb	*Barbus ticto*	F?	1 (30d)	highly toxic	AQUIRE (Upadhyaya & Rao, 1980)
Brown trout	*Salmo trutta*	?	70	slightly toxic	Grande et al., 1994
Brown trout	*Salmo trutta*	F?	83	slightly toxic	AQUIRE (Aanes, 1992)
Mozambique tilapia	*Tilapia mossambica*	F?[c]	3.1	moderately toxic	Rao & Dad, 1979
teleost	*Punctius ticto*	F?[c]	24.5	slightly toxic	Rao & Dad, 1979
teleost	*Danio sp.*	F?[c]	12.6	slightly toxic	Rao & Dad, 1979

a. The 24-hour LC50 for coho salmon was reported as 11 ppm.
b. WP= wettable powder; WDG= water dispersible granules
c. Identified as "Tafazine", which probably means it was a 50% WP

(2) Acute toxicity to freshwater invertebrates

Results from acute studies with freshwater invertebrates (Table 4) indicate that technical grade simazine is, at most, moderately toxic to several freshwater invertebrates. It appears to be considerably less toxic to a number of other aquatic invertebrates, however as with fish, the solubility limit of simazine is such that tests with technical material indicating LC50 or EC50 values above about 16 ppm (or less if not "solubilized") are suspect; but at the same time, these data indicate a low likelihood of any simazine lethality in the natural environment. The lowest acute invertebrate test is considered to be 1.1 ppm for *Daphnia magna*.

Invertebrates serve as a food source for juvenile salmon and steelhead. Comparative toxicology of various invertebrate species is important because a reduction in a single species may not be relevant unless it is an abundant and key food source., whereas reductions in many species or key species may be very relevant. It appears that, based either upon reported LC50/EC50 values or the solubility of simazine, effects are likely to be different for various aquatic invertebrates that might serve as food sources for listed salmon and steelhead..

Table 9. Acute toxicity of simazine to freshwater invertebrates.

Species	Scientific name	% a.i.	96-hour LC50 (ppm)	Toxicity Category	Reference
Scud	*Gammarus fasciatus*	98.1	130[a]	practically non-toxic	EFED (Mayer & Ellersieck, 1980)
Stonefly	*Pteronarcys californica*	98.1	1.9	moderately toxic	EFED (Johnson & Finley, 1980)
Water flea	*Daphnia magna*	98.1	1.1[a] (48 hr)	moderately toxic	EFED (Johnson & Finley, 1980)
Water flea	*Daphnia magna*	98.1	>10 (48 hr)	$\leq$ slightly toxic	EFED (Mayer & Ellersieck, 1986)
Seed shrimp	*Cypridopsis vidua*	98.1	3.7[a] (48 hr)	moderately toxic	EFED (Johnson & Finley, 1980)
Aquatic sowbug	*Asellus brevicauda*	tech	>100 (48 hr)	practically non-toxic	EFED
Crayfish	*Procambarus sp.*	tech	>100 (48 hr)	practically non-toxic	EFED
Glass shrimp	*Palaemonetes kadiakensis*	tech	>100 (48 hr)	practically non-toxic	EFED (Mayer & Ellersieck, 1986)
formulated product[b]					
Water flea	*Daphnia magna*	80WP	>10 (48 hr)	$\leq$ slightly toxic	EFED (Mayer & Ellersieck, 1986)
Scud	*Gammarus lacustris*	WP %unk	13	slightly toxic	EFED (Sanders, 1969)
Water flea	*Daphnia pulex*	80WP	92.1 (48 hr)	slightly toxic	Fitzmayer et al., 1982
Midge	*Chironomus tentans*	50	3.58 (48 hr)	moderately toxic	Dad & Tripathi, 1980
Unidentified or inadequately identified material					
Water flea	*Daphnia pulex*	F?	3 hr >40	slightly toxic	AQUIRE (Nishiuchi & Hashimoto, 1967)
Water flea	*Moina macrocarpa*	F?	3 hr >40	slightly toxic	AQUIRE (Nishiuchi & Hashimoto, 1967)

[a] It appears that Sanders (1970) was the original source of these data, since they were all conducted at the Columbia National Fisheries Laboratory. However, Sanders (1970) reported LC values of 1.0 ppm for *Daphnia magna*, 3.2 for *Cypridopsis vidua* and >100 ppm for *Gammarus fasciatus*. Mayer & Ellersieck (1986) and Johnson & Finley (1980) may have adjusted the LC50 values to reflect the percentage of active ingredient, although this was not stated, and we cannot be sure. However, such an adjustment would account for the relatively minor differences.
b WP= wettable powder; WDG= water dispersible granules

(3) Chronic toxicity to freshwater fish and invertebrates

The chronic toxicity data for simazine are limited to tests with the 80% wettable powder formulation, the formulation registered for aquatic use. These tests were conducted at concentrations up to 2.5 ppm. For the bluegill, goldfish, and *Daphnia magna*, no effects were observed at this concentration. There were effects on fry growth of fathead minnows at 2.5 ppm; the no-observed-effect-concentration was 1.2 ppm.

Table 10. Chronic toxicity of simazine to freshwater fish and invertebrates.

Species	Scientific name	Duration	% a.i.	Endpoints affected	NOEC (ppm)	LOEC (ppm)	Reference
Fathead minnow	*Pimephales promelas*	120 d	80WP	fry growth	1.2[a]	<2.5	EFED
Bluegill sunfish	*Lepomis macrochirus*	360 d	80WP	none observed	2.5	>2.5	EFED
Goldfish	*Carassius auratus*	360d	80WP	none observed	2.5	>2.5	EFED
Water flea	*Daphnia magna*	21 d	80WP	none observed	2.5	>2.5	EFED
Water flea	*Daphnia pulex*	26d	80WP	adult survival[b]; #young	<4.0	4	Fitzmayer, et al., 1982

a. This should be considered the NOAEC, i.e., the no-adverse-effect-concentration. The actual NOEC level is 0.31 ppm, but the effect is increased hatchability and is considered a beneficial effect; for regulatory purposes, the 1.2 ppm level is considered as the NOEC.
b. 65% adult mortality at 4 ppm (lowest concentration tested) after 26 days.

(4) Acute toxicity to estuarine and marine fish

The very limited acute test results indicate that technical grade simazine and the 80% wettable powder formulation have LC50 values exceeding the limit of solubility (Table 7).

Table 11. Acute toxicity of simazine to estuarine and marine fish.

Species	Scientific name	% a.i.	96-hour LC50 (ppm)	Toxicity Category	Reference
Striped bass	*Morone saxatilis*	80WP	> 3	≤ moderately toxic	EFED
Sheepshead minnow	*Cyprinodon variegatus*	96.9	> 4.3	≤ moderately toxic	EFED

(5) Acute toxicity to estuarine and marine invertebrates

Acute toxicity tests with estuarine and marine invertebrates (Table 12) indicate that technical grade simazine is no greater than moderately toxic to oysters and is not toxic to the tested crab and shrimp at the limit of solubility (the 113 ppm point is well above solubility limits).

Table 12. Acute toxicity of simazine to estuarine and marine invertebrates.

Species	Scientific name	% a.i.	96-hour LC50 (ppm)	Toxicity category	Reference
Mud crab	*Neopanape texana*	98.1	>1000	practically non-toxic	EFED
Pink shrimp	*Penaeus duorarum*	98.1	113	practically non-toxic	EFED
Eastern oyster (weight gain)[a]	*Crassostrea virginica*	99.1	> 1 (7-day)	≤ moderately toxic	EFED
Eastern oyster (shell deposition)	*Crassostrea virginica*	96.9	> 3.7[b]	≤ moderately toxic	EFED

a. The test with weight gain as the endpoint is a non-standard test and should not be compared to other test data on oysters. There was approximately a 50% inhibition in weight gain at 1ppm
b. There was 6.8% shell-growth inhibition at the highest concentration

(6) Chronic toxicity to estuarine and marine fish and invertebrates

There are no data available on the chronic toxicity of simazine to estuarine and marine organisms. The acute toxicity data for estuarine and marine organisms are mostly “greater than” data that do not provide much information on relative sensitivity of freshwater versus estuarine/marine species. However, there are no acute data that suggest simazine will be more toxic to estuarine and marine organisms, on a chronic basis, than it is to freshwater organisms. Our best conservative judgement is that chronic no-effect levels to estuarine/marine fish would be 1 ppm or greater. The standard estuarine/marine invertebrate is often considerably more sensitive than freshwater arthropods to the point that we cannot make any assumptions about chronic effect or no-effect levels.

(7) Toxicity to aquatic plants and algae

There is a modest amount of data on aquatic vascular plants. There is an exceedingly large amount of data on algae, probably because simazine is used as an algacide. We have not attempted to report all algae data, just those that we believe represent relevant sensitivity. Both the vascular plant and algae data are presented in Table 13.

In addition, there are data that vary considerably from standardized tests, or that otherwise warrant additional discussion. Merlin et al., (1993) reported apparently anomalous results for Lemna growth where the 4-day EC50 was lower (350 ppb) than the 10-day EC50 (550 ppb). They attributed this to the adaptation of the *Lemna* to increase photon capture efficiency. Faust et al., (1993) investigated the toxicity of simazine to the algae *Chlorella fusca* in combination with other pesticides: atrazine, bentazon, chlorotoluron, 2,4-D acid, glyphosate, metazachlor, and triallate. For all simazine combinations, they reported that the toxicity was additive.

Peterson, et al. (1994) tested a single concentration of 2.667 mg/l of simazine (Princep formulation 80%) with the duckweed, *Lemna minor*, 4 algal species, and 6 cyanobacteria species. The test concentration was based on calculated EEC that would result from direct application of 4 kg/Ha to six inches of water. *Lemna* were tested for seven days, the algae and cyanobacteria were incubated for 22 hours. At this concentration, there was 100% inhibition of growth, relative to controls, for the *Lemna*, and 63-99% growth inhibition for the various algae and cyanobacteria.

Wilson, et al. (2000) investigated the uptake and toxicity of simazine to cattails (*Typha latifolia*). They reported a no-observed-effect-concentration of 0.3 mg/l, and a lowest-observed-effect-concentration of 1 mg/l. Based on the uptake studies where 65% of the simazine was removed from the test medium, they suggested that cattails can be used as a phytoremediation tool to reduce simazine concentrations.

Table 13. Acute toxicity of simazine to algae and vascular plants.

Species	Scientific name	% a.i.	length (days)	EC50 (ppb)	Reference
Green algae	*Dunaliella tertiolecta*	98	10	5000	EFED
Green algae	*Chlorococcum sp.*	98	10	2000	EFED
Marine algae	*Isochrysis galbana*	98	10	500	EFED
Green algae	*Selanastrum capricornutum*	96.9	5	100	EFED
Green algae	*Selanastrum capricornutum*	tech	4	1240	Fairchild et al., 1997
Blue-green algae	*Anabaena flos-aquae*	96.9	5	36	EFED
Freshwater diatom	*Navicula pelliculosa*	96.9	5	90	EFED
Marine diatom	*Skeletonema costatum*	96.9	5	600	EFED
Marine diatom	*Phaeodactylum tricornutum*	98	10	500	EFED
Green algae	*Scenedesmus sp.*	90WP	?	59	Wall, 2003
Green algae	*Chlorella fusca*	98	1	73	Faust et al., 1993
Duckweed	*Lemna gibba*	96.9	14	140	EFED

Duckweed	*Lemna minor*	NR	10	550	Merlin et al., 1993
Duckweed	*Lemna minor*	NR	4	350	Merlin et al., 1993
Duckweed	*Lemna minor*	tech	4	166	Fairchild et al., 1997

(8) Other simazine toxicity data

Walker (1964) conducted several kinds of studies with simazine because of its proposed use as an aquatic herbicide. Results were not reported numerically, but rather were graphed in a fashion that precludes an accurate estimation of LC50 or other results data. However, he reported several relevant items.

With respect to formulation toxicity to fish (various species of *Lepomis*), he found that the granular formulation with ammonium sulfate was the most toxic to fish, whereas the granular formulation with calcium sulfate was the least toxic. The wettable powder was the second most toxic, while granules with attapulgite clay, in 8% and 4% formulations were less toxic than the wettable powder.

The most sensitive “bottom fauna” organism was the midge, with an “LD50" of 28 ppm, and aquatic oligochaete worms were comparably sensitive. He indicated that the limited solubility of simazine resulted in a layer of precipitated simazine on top of the bottom mud in ponds when higher concentrations were used.

The purpose of Walker’s research was for evaluating control of aquatic weeds; consequently the data from field tests on these weeds was expressed in terms of “control.” At rates of 1-2 ppm, rice cut-grass (*Lersia oryzoides*), bulrush (*Scirpus validus* & *Scirpus americanus*), needlerush (*Eleocharis acicularis*), arrowhead (*Sagitarria latifolia*), water plantain (*Alisma subcordatum*), ripgut (*Carix riparia*), smartweed (*Polygonum* sp.), water primrose (*Jussiaea* sp.), and willow (*Salix* sp) were “controlled”. “Tolerant” plants at these concentrations include American lotus (*Nelumbo lutea*), cattails (*Typha latifolia*, *Typha angustifolia*), sweetflag (*Acorus calams*), and duckweed (*Lemna minor*, *Spirodela polyrhiza = Lemna major*). Higher rates of 4-12 ppm produced partial or temporary control of these species.

Walker concluded that there were no effects on the fish fauna from any of the treatments under field conditions, nor was there any oxygen depletion as a result of the weed control.

(9) Toxicity of degradates

Syngenta, the primary registrant of simazine, submitted endpoint toxicity data for metabolites of simazine (Wall, 2003). These were developed according to standard protocols, but they have not been reviewed by EFED or validated (Table 14). Most of these data indicate slight toxicity or less. However, the *Scenedesmus* exhibits moderate toxicity with G28279, and the *Scenedesmus* with GS17791 and the rainbow trout with GS17792 may be as much as moderately toxic.

Table 14. Acute toxicity of simazine degradates.

Species	Scientific name	metabolite	length (days)	EC50 (ppm)	category	Reference
Green algae	*Scenedesmus subspicatus*	G30414	3	>100	practically non-toxic	Wall, 2003
Rainbow trout	*Oncorhynchus mykiss*	G28279	4	17.2	slightly toxic	Wall, 2003
Water flea	*Daphnia magna*	G28279	2	126	practically non-toxic	Wall, 2003
Green algae	*Scenedesmus subspicatus*	G28279	3	1.39	moderately toxic	Wall, 2003
Rainbow trout	*Oncorhynchus mykiss*	GS17792	4	>8.4	≤ moderately toxic	Wall, 2003
Water flea	*Daphnia magna*	GS17792	2	>26.7	≤ slightly toxic	Wall, 2003
Green algae	*Scenedesmus subspicatus*	GS17792	3	>28.8	≤ slightly toxic	Wall, 2003
Rainbow trout	*Oncorhynchus mykiss*	G28273	4	>100	practically non-toxic	Wall, 2003
Water flea	*Daphnia magna*	G28273	2	>100	practically non-toxic	Wall, 2003
Green algae	*Scenedesmus subspicatus*	G28273	3	>100	practically non-toxic	Wall, 2003
Rainbow trout	*Oncorhynchus mykiss*	GS17791	4	11	slightly toxic	Wall, 2003
Water flea	*Daphnia magna*	GS17791	2	>14.2	≤ slightly toxic	Wall, 2003
Green algae	*Scenedesmus subspicatus*	GS17791	3	>6.3	< moderately toxic	Wall, 2003

(10) Toxicity of inerts

There are no available data on the toxicity of "inert" ingredients in simazine products. However, there are reliable data on four different formulated products. These data provide no indication that ingredients other than active ingredients contribute noticeably to the acute toxicity. While the data show the formulations to be less toxic, in general, quantitative comparisons may be inappropriate because of the relatively low solubility of simazine, especially as the technical material. The single test on the granular product (Simazine 4G) with fathead minnows does show somewhat more toxicity than does the technical material, but the difference is well within the two-fold range expected for intralaboratory testing, and is considered insignificant relative to the four-fold difference expected for interlaboratory testing.

(11) Review of literature on sublethal and endocrine effects

Other than the report of signs of toxicity in the standard acute and chronic toxicity tests, we are not aware of any tests dealing specifically with sublethal effects of simazine. We are also not aware of any data that indicates that simazine disrupts endocrine functions, except as may have occurred in the reproductive effects tests. We do note that simazine is a triazine herbicide, and at least one triazine herbicide, atrazine, is considered to be an "endocrine disruptor" at certain concentrations, which have not been well defined for aquatic organisms.

b. Environmental fate and transport

Some of the data requirements have been satisfied when simazine registrations were sought in the past, and there are some additional valid studies that do not satisfy guideline requirements. However, we are unaware of any comprehensive evaluation of these fate and transport data as a whole. Below we are presenting the results of relevant individual studies that have been submitted and validated by OPP, followed by a brief summary of key characteristics.

Simazine was stable to hydrolysis at pH 5, 7, and 9 for 28 days. It is concluded that hydrolysis is not an important degradation mechanism for simazine. The aqueous photolysis half-life is greater than 30 days in sterile water; however, when a photosensitizing agent was added, the half-life was about 1 day. Humic acids are natural photosensitizing agents that may be present in some waters, especially in forested areas and other areas with decaying organic matter. Degradates identified in the aqueous photolysis study are G-28273, G-30414 and GS-17792.

The soil photodegradation half-life was 207-234 hours on sandy loam soil; identified degradates, which did not exceed 10%, were 2-amino-4-chloro-6-ethylamino-s-triazine (G-28279), 2,4-bis(ethylamino)-6-hydroxy-s-triazine (G-30414), 2-amino-4-ethylamino-6-hydroxy-s-triazine (GS-17792), and 2,4-diamino-6-s-triazine (G-28273). The soil aerobic metabolism study showed a half-life of 110 days; the metabolites were G-28279, G-30414, G-28273, GS-17792, G-28516 and GS-17791. In flooded anaerobic soils, the metabolism half-life was about 8 weeks; identified metabolites were G-28279, G-30414, G-28273, and GS-17792.

The anaerobic aquatic metabolism study showed that simazine degraded with a half-life greater than 365 days in sandy clay sediment flooded with pond water; degradates were G-30414, G-28279, and G-28273. The calculated half-life for aerobic aquatic metabolism in the dark was 61.3, 108.8, and 71.2 days for the sediment extracts, water layer, and overall, respectively. The degradates identified were G-28279, G-30414, G-30044, and G-31709.

Simazine is mobile, i.e., not strongly adsorbed onto soil; Kd constants for the adsorption phase were below 5 (range 0.48-4.31) for all tested soils. Desorption constants varied from 0.78 for a loam soil to 9.34 for a clay soil. The sorption constants correlated, in general, with organic matter content. The hydroxysimazine degradate (G-30414) was less mobile than the parent simazine, and the dealkylated degradates G-28273 and G-28279 were more mobile and more likely to leach than simazine.

Terrestrial field dissipation studies do not satisfy guideline requirements, but they do provide some information. Bare ground studies indicated dissipation (i.e., degradation plus transport from the top 6-8 inches of soil) half-lives of 186 days in Minnesota, 149 days in Ripon, California, and 87 days in Florida. In another Florida study on bare ground and on a citrus crop, dissipation half-lives were 33 and 44 days, respectively. In an Oregon study, on bare ground and raspberries, dissipation half-lives were 125 and 119 days, respectively. In Missouri with bare ground and corn, dissipation half-lives were 101 and 110 days, respectively. In all studies, various degradates were found at various depths in the soil profiles below 6 inches. In general, residues of degradates in the 6-8 inch surface layer were less than 10% of the parent; however, the degradate G28279 was found at about 25% of the applied simazine in the Oregon study.

Simazine has a low potential to bioaccumulate in rainbow trout. The BCF was 0.9-2.3x in viscera and muscles. The depuration half-life was about 28 days. The degradates G-28279 and G-28273 also exhibited a very low potential for bioconcentration.

A summary of simazine degradates is in Table 15. There are two major types of degradates for simazine. The first type of degradates are formed via dealkylation of the amino groups, for which mono- and fully dealkylated degradates are known. The second type of degradates are formed by substitution of a chloro group by a hydroxy group in either parent or dealkylated degradates.

Table 15. Summary of simazine degradates detected in the laboratory studies.

Major Degradates	Photolysis in Water	Photolysis on Soil	Aerobic Soil	Anaerobic Soil	Aquatic Anaerobic	Aquatic Aerobic
G-30414	X	X	X	X	X	X
G-28279		X	X	X	X	X
G-28273	X	X	X	X	X	X
GS-17792	X	X	X	X		
GS-28516			X	X		
GS-17791			X			
G-31709						X
G-30044						X

In summary, simazine is fairly persistent and mobile. Degradation appears to result most quickly by photodegradation on soil surfaces and through microbial transformation in water. Degradates are either not toxic or are not formed in sufficient quantity to be of concern relative to the parent simazine. Both simazine and most of its degradates are likely to leach. There is no potential for bioaccumulation.

c. Incidents

There are five reported incidents where simazine was the apparent cause of fish mortality. Specific application information is lacking. These incidents occurred from 1989-1992. In one, bluegill and catfish were thought to be killed by application of simazine to corn; this kill is considered "unlikely" to have been caused by simazine. In a second, an unknown number of several fish species were killed in Nebraska apparently from simazine use on a railroad right-of-way. This kill is considered "possible." This kill occurred before there was a 10-fold rate reduction for this use, but it is not known what the actual rate was. Three incidents occurred from aquatic use of simazine in a pond in Tennessee and in a lake and a pond in California. The ponds were not described and could have been ornamental ponds, a currently registered use, or natural ponds, where simazine is not currently registered for use. Simazine is not registered for use in any kind of lake, and it does not appear that it was in the past.

d. Estimated and actual concentrations of simazine in water

(1) EECs from models

The refined tier II approach with PRZM/EXAMS was used for simazine. The upper tenth percentile concentration values, expressed in ppb (ug/L), are summarized in Table 16 below. The results of three uses, corn, citrus, and apple, were based on the standard scenarios intended to predict reasonable high exposure values, i.e., soils with high runoff potential and heavy rainfall amounts.

Table 16. Maximum EECs (ppb) of simazine from selected application scenarios.

Use	rate (#ai/A)	no. of appl.	Peak	96-hr average	21-d average	60-d average	90-d average
Corn	4	1	48.67	48.15	46.16	41.82	38.5
Citrus	4	2	112.2	110.9	107.3	98.28	52.26
Apple	4	1	30.66	30.1	28.67	25.97	24.75

These EECs are used with the toxicity data to assess acute and chronic risks to freshwater organisms based upon the farm pond model for the receiving water. Acute risks are assessed using peak EECs. Chronic risk quotients are assessed using the appropriate long term averages. As we have stated in previous requests, the EECs based upon high rainfall eastern areas overstate the EECs that would be expected in the more arid western states that should be used for Pacific salmon and steelhead; in addition the farm pond model would not necessarily relate to flowing water situations, except for acute exposures in first order streams.

Several uses of simazine are not amenable to modeling for EECs. However, the rates for terrestrial uses are similar to those that have been modeled and there is no basis suggesting that they would result in higher EECs than those modeled for the citrus use. The aquatic uses are for closed systems that should not enter any natural waters where listed salmon and steelhead occur. In addition, the fact that simazine is used in fish husbandry, tropical fish aquaria, and garden fish ponds demonstrates the lack of direct effects on fish.

(2) Measured residues in the environment

Simazine has been subject to monitoring in the NAWQA program; there are several thousands of samples taken. The highest residue level found in the western U. S. was less than 40 ppb.

Troiano and Garretson (1998) investigated simazine runoff in citrus orchards in the southern San Joaquin Valley. They looked at applications at a rate of 2.2Kg/Ha applied to the middle of the rows between trees, an area where farm vehicles often compacted the soils. They compared the undisturbed compacted soils with soils that had been mechanically disturbed to break up the compacted layer. They then applied 3.2 cm of “artificial rain”and measured simazine only in the water running off the orchard, not in any kind of receiving water. The maximum residues in the runoff water were 870 ppb for the compacted soils and 140 ppb for the disturbed soils. After a second “artificial rain” of another 3.2 cm a week later, maximum residues in the runoff water were 400 ppb and 70 ppb for the compacted and disturbed soils, respectively. They concluded that infiltration into the disturbed soil layer was responsible for the difference.

Powell et al. (1996) looked at the residues in runoff water for simazine applied to roadside rights-of-way in Glenn County, California. They applied 2.02 Kg/Ha of simazine, along with diuron, to a 2.4 M wide strip on the shoulders of a paved highway. Simulated rain (13 mm in 1 hr) was applied to plots on treated highway shoulders at three sites. At one site, none of the artificial rainfall ran off the plot. At the other two sites, 5-12% and 17-46% of the applied water ran off. Simazine concentrations in the runoff water at these two sites, respectively, ranged from 78-447 and from154-574 ug/L. Natural runoff from one quadrant of a freeway interchange where simazine was applied also was sampled during several storms from a flume that discharged runoff into a drainage canal. The first runoff sample was taken after a total of 100 mm of rain had fallen, and simazine concentration averaged 105 ug/L, which was higher than subsequent samples.

Both the rights-of-way and orchard studies measured simazine only in the runoff water. Any simazine residues in receiving water would depend on the nature of that receiving water. However, there should be significant dilution. It is exceedingly rare that rain would fall only on treated portions of rights-of-way or orchards. Therefore, even the runoff water from an entire area would provide substantial dilution to the simazine in the runoff water. Additional dilution would occur as a result of water already present in the ditch, pond, or other body of water into which the runoff would flow. Since our LOC for direct effects is 320 ppb and for indirect effects is 140 ppb, there is negligible or no potential for these to be exceeded by the time any such runoff water reaches areas where salmon and steelhead would occur.

e. Recent changes in simazine registrations

There have been few recent changes in simazine registrations. A Reregistration Eligibility Decision document is not expected until 2004, and it is during the period when a pesticide is undergoing reregistration review that changes are most likely to occur. Should there be any changes prior to issuance of a biological opinion, we will transmit that information to the Service.

We do need to note that the changes that occurred in approximately 1997 resulted in a reduction of application rates for non-crop industrial and rights-of-way uses of simazine. Older labels had rates of up to 40 lb ai/A for these uses. Newer labels now have a maximum rate of 4.8 lb ai/A. All products in use have the newer, lower non-crop rate. There is one product with the older, higher rate that is technically still registered, but it has not been marketed since the 1980s, and the 1985 label is not in compliance with the 1992 Worker Protection Standards (amended 1995) for pesticides; as a result, marketing of this old product would be a violation of these standards.

f. Existing protections

Nationally, there are no specific protective measures for endangered and threatened species beyond the generic statements on the current simazine labels. The Environmental Hazards Statement for simazine is:

> Do not apply directly to water, to areas where surface water is present, or to intertidal areas below the mean high water mark. Do not contaminate water by cleaning of equipment or disposal of wastes. Simazine is a chemical which can

travel (seep or leach) through soil and enter ground water which may be used as drinking water. Simazine has been found in ground water as a result of its use a herbicide. Users of this product are advised not to apply simazine where the water table (ground water) is close to the surface and where the soils are very permeable, i.e., well-drained soils, such as loamy sands. Users are advised to consult with their local agricultural agencies to obtain information on the location of ground water and the type of soil in their area.

OPP's endangered species program has developed a series of county bulletins which provide information to pesticide users on steps that would be appropriate for protecting endangered or threatened species. Simazine is included in some county bulletins for the protection of T&E plants. It is not included for aquatic animals, and based on this current analysis does not need to be included for listed salmon and steelhead, and perhaps not for any listed aquatic animal, although species-specific analyses would be required to substantiate that.

g. Discussion and conclusions for simazine

(1) Fish

The lowest fish LC50 for technical simazine is 6.4 ppm for fathead minnow. OPP's level of concern for endangered species is 0.05 times the LC50. Thus, OPP would consider endangered fish to be at acute risk when simazine concentrations exceed 320 ppb. The chronic no-observed-effect-concentration for fish is considered to be 1.2 ppm. Using the no-effect criterion for acute toxicity provides an additional safety margin, relative to the chronic NOEC, for any potential chronic effects. In addition, there is a very low likelihood of continuous exposure over a length of time to reflect chronic effects.

(2) Invertebrates

The most sensitive aquatic invertebrate acute study is an EC50 of 1.1 ppm for *Daphnia magna*. OPP's criteria consider that an EEC greater than 0.5 times the LC50 could have an effect on populations of aquatic invertebrates that may serve as a food source for listed fish. On this basis, concerns for indirect effects on the food supply for fish (including threatened and endangered salmonids) would occur at concentrations greater than 550 ppb. The chronic NOEC for *Daphnia magna* is 2.5 ppm. Using the 350 ppb LOC for direct, acute effects on T&E fish will protect the invertebrate food supply for these fish.

(3) Cover

The most sensitive aquatic vascular plant test data is an EC50 of 140 ppb for *Lemna gibba*. OPP's criteria consider that an EEC greater than the EC50 could have an effect on populations of aquatic plants that may serve as cover for listed fish.

(4) Discussion and conclusions

Levels of concern for simazine are 320 ppb for acute direct effects on T&E salmon and steelhead, 550 ppb for the invertebrate food supply, and 140 ppb for aquatic plant cover. The chronic concern level for fish is 1200 ppb. The highest EEC is 112.2 ppb for 2 applications of 4 lb ai/A in Florida citrus. Florida citrus is intended to yield the highest EECs nationally for citrus. California citrus should be much less. In addition, the Florida scenario is for two applications of 4 lb ai/A, whereas the maximum label rate in California is one application of 4 lb ai/A. No other use, crop or non-crop, has an application rate higher than 5 lb ai/A. Because the high rainfall, high runoff Florida citrus EEC at twice the western citrus rate, and higher than western rates for any other crop or non-crop use, is still well below any of the criteria of concern, I conclude that there will be no effect of simazine on the T&E Pacific salmon and steelhead. As a result, I am not including the typical information on individual ESUs; all will be below our levels of concern.

4. References

Bathe R, Sachsse K, Ullmann L, Hormann WD, Zak F, Hess R. 1975. The Evaluation of Fish Toxicity in the Laboratory. Proc.Eur.Soc.Toxicol. 16:113-124

Beyers DW, Keefe TJ, Carlson CA. 1994. Toxicity of carbaryl and malathion to two federally endangered fishes, as estimated by regression and ANOVA. Environ. Toxicol. Chem. 13:101-107.

Bilby RE, Fransen BR, Bisson PA, Walter JK. 1998. Response of juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead (*Oncorhynchus mykiss*) to the addition of salmon carcasses to two streams in southwestern Washington, U. S. A. Can. J. Fish. Aquat. Sci. 55:1909-1918.

Bills TD, Marking LL, Howe GL.1993. Sensitivity of Juvenile Striped Bass to Chemicals Used in Aquaculture. Resour. Publ.192, Fish Wildl.Serv., U.S.D.I., Washington, D C.

Bond CE, Lewis RH, Fryer JL. 1960. Toxicity of various herbicidal materials to fishes. Pp96-101 *in* Tarzwell CM (ed.) Biological problems in water pollution. Trans. 1959 Semin., Robert A. Taft Sanit. Eng. Cent. Tech Rept W60-3.

Dad NK, Tripathi PS. 1980. Acute toxicity of herbicides to freshwater fish and midge larvae, *Chironomus tentans*. Environment International, 4:435-437.

Dwyer FJ, Hardesty DK, Henke CE, Ingersoll CG, Whites GW, Mount DR, Bridges CM. 1999. Assessing contaminant sensitivity of endangered and threatened species: Toxicant classes. U.S. Environmental Protection Agency Report No. EPA/600/R-99/098, Washington, DC. 15 p.

Effland WR, Thurman NC, Kennedy I. 1999. Proposed Methods For Determining Watershed-Derived Percent Cropped Areas and Considerations for Applying Crop Area Adjustments To Surface Water Screening Models. USEPA Office of Pesticide Programs. Presentation to FIFRA Science Advisory Panel, May 27, 1999.

Fairchild JF, Ruessler DS, Haverland , Carlson AR.1997. Comparative Sensitivity of *Selanastrum capricornutum* and *Lemna minor* to Sixteen Herbicides. Arch. Environ. Contam. Toxicol. 32:353-357.

Faust M, Altenburger R, Boedeker W, Grimme LH. 1993. Additive effects of herbicide combinations on aquatic non-target organisms. Sci.Total Environ.(Suppl.):941-951.

Fitzmayer KM, Geiger JG, Van den Avyle MJ.1982. Effects of Chronic Exposure to Simazine on the Cladoceran, *Daphnia pulex*. Arch.Environ.Contam.Toxicol. 11(5):603-609.

Grande M, Andersen S, Berge D. 1994. Effects of pesticides on fish: experimental and field studies. Norwegian Journal of Agricultural Sciences, Supplement 13:195-209.

Johnson WW, Finley MT. 1980. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. USFWS Publication No. 137.

Kansas State University. 1990. Herbicide Mode of Action. Publication C-715, Cooperative Extension Service, Kansas State University, Manhattan, KS.

Mayer FL. 2002. Personal communication, Foster L.Mayer Jr., U.S.EPA, Environmental Research Laboratory, Gulf Breeze, Florida. August 2002.

Mayer, FL, Mayer KS, Ellersieck MR. 1986. Relation of survival to other endpoints in chronic toxicity tests with fish. Environ Toxicol Chem 5:737-748.

Mayer FL, Ellersieck MR, Krause GF, Sun K, Lee G, Buckler DR. 2002. Time-concentration-effect models in predicting chronic toxicity from acute toxicity data. Pages 39-67 *in* M Crane, MC Newman, PF Chapman, and J Fenlon, eds. Risk Assessment with Time to Event Models. Boca Raton, FL.

Merlin G, Eulaffroy P, Blake G. 1993. Use of Fluorescence Induction Kinetics of Lemna minor as a Tool for Chemical Stress Evaluation. Sci.Total Environ.(Suppl.):761-772

Nishiuchi Y, Hashimoto Y. 1969. Toxicity of Pesticides to Some Fresh Water Organisms. Rev. Plant Protec. Res. 2:137-139.

Peterson HG, Boutin C, Martin PA, Freemark KE, Ruecker NJ, Moody MJ. 1995. Aquatic phyto-toxicity of 23 pesticides applied at expected environmental concentrations. Aquat. Toxicol., 28:275-292.

Powell S, Neal R, Leyva J. 1996. Runoff and leaching of simazine and diuron used on highway rights-of-way. Report EH 96-03, Environmental Monitoring and Pest Management Branch, California Department of Pesticide Regulation.. Executive summary on-line at: http//www.cdpr.ca.gov/docs/empm/pubs/ehapreps/e9603.htm

Rao KS, Dad NK.1979. Studies of Herbicide Toxicity in Some Freshwater Fishes and Ectoprocta. J.Fish Biol. 14(6):517-522.

Sanders HO.1969. Toxicity of Pesticides to the Crustacean Gammarus lacustris. Tech. Pap. No. 25, Bur. Sports Fish.Wildl., Fish Wildl. Serv., U.S.D.I., Washington, D.C. :18 p.

Sanders HO. 1970.   Toxicities of some herbicides to six species of freshwater crustaceans. Jour. Water Poll Ctrl Fed. 42(8), part 1: 1544-1550.

Sappington LC, Mayer FL, Dwyer FJ, Buckler DR, Jones JR, Ellersieck MR. 2001. Contaminant sensitivity of threatened and endangered fishes compared to standard species. Environ. Toxicol. Chem. 20:2869-2876.

TDK Environmental. 2001. Diazinon & Chlorpyrifos Products: Screening for Water Quality. Contract Report prepared for California Department of Pesticide Regulation. San Mateo, California.

Troiano J and Garretson C. 1998. Movement of Simazine in Runoff Water from Citrus Orchard Row Middles as Affected by Mechanical Incorporation. J. Environ. Qual. 27:488-494.

Tucker RK, Leitzke JS. 1979. Comparative toxicology of insectides for wildlife and fish. Pharmacol. Ther. 6:167-200.

Urban DJ, Cook NJ. 1986. Hazard Evaluation Division - Standard Evaluation Procedure - Ecological Risk Assessment. U.S. EPA Publication 540/9-86-001.

U. S. Department of Agriculture. 2002. Agricultural Chemical Usage: 2001 Fruit Summary, USDA National Agricultural Statistics Service. Online at: http://usda.mannlib.cornell.edu/reports/nassr/other/pcu-bb/#fruits

U. S. Department of Agriculture. 2002. Agricultural Chemical Usage: 2000 Nursery and Floriculture Summary, USDA National Agricultural Statistics Service. Online at: http://usda.mannlib.cornell.edu/reports/nassr/other/pcu-bb/#nursery.

Wall SB. 2003. Information in support of simazine use in the western United States relative to endangered salmonid species. Syngenta Number 1216-03, Syngenta Crop Protection, Inc., Greensboro, NC. 19p.

Walker CR. 1964. Simazine and other s-triazine compounds as aquatic herbicides in fish habitats. Weeds, 2(2):134-139.

Wilson PC, Whitwell T, Klaine SJ. 2000. Metalaxyl and simazine toxicity to and uptake by *Typha latifolia*. Arch. Environ. Contam. Toxicol., 39:282-288.

Zucker E. 1985. Hazard Evaluation Division - Standard Evaluation Procedure – Acute Test for Freshwater Fish. U.S. EPA Publication 540/9-85-006.